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14. ABSTRACT

Prostate carcinoma is the most common form of cancer in American men. The etiology of prostate cancer is currently unknown. It is known that during progression from low to high-grade carcinoma, the spectrum of cytologic, biological, and genetic features changes. We believe that these changes may be a result of defects in the centrosome, an essential organelle that organizes spindle poles during mitosis and has important roles in cell proliferation, cell polarity, and genetic stability. We have shown that centrosomes are defective in prostate carcinoma and pre-invasive lesions. We also showed that the essential centrosomal protein pericentrin is elevated in both pre-invasive prostate lesions and invasive prostate tumors, induces cancer like lesions when overexpressed and binds AKT, PKA and PKC. In this proposal we will follow up on these observations by: examining pericentrin's oncogenic potential after disrupting its interactions with the kinases AKT, PKA and PKC and determine whether other oncogenes or tumor suppressors act synergistically or antagonistically with pericentrin in prostate cancer.

15. SUBJECT TERMS
Centrosome defects, genetic instability, prostate cancer progression

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Introduction

The work in this proposal is designed to investigate the role of pericentrin as an oncogene in prostate cancer. Pericentrin is a centrosome protein involved in organizing mitotic spindles to ensure proper chromosome segregation (Doxsey et al, 2005; Zimmerman et al, 2004). Centrosomes are tiny cellular organelles that duplicate once per cell cycle and the pair of centrosomes forms the two poles of the mitotic spindle during cell division. Each centrosome is comprised of two microtubule barrels called centrioles. Our previous work and that of another group was the first to show that centrosome defects were present in nearly all prostate cancers (Pihan et al, 1998; Lingle et al, 1998) and that centrosome defects were present in preinvasive lesions of the prostate (Pihan et al, 2003). Other studies from our group showed that pericentrin overexpression induced features of prostate cancer cells including aneuploidy, centrosome and spindle defects, increased growth in soft agar and changes in cell morphology (Pihan et al, 2001). To expand on these observations, we are currently testing whether other known oncogenes and tumor suppressors contribute to the pericentrin-induced phenotype.

Body

Katryn Harwood initiated the work described in this proposal. She left the Doxsey laboratory and I, Chun-Ting Chen, have taken over the project in the last few months. Over the last year, Kathryn and I have made progress on both Aims of this proposal. I have focused on cell lines that I had been using before the grant transfer (HeLa, SAOS, RPE). The Doxsey lab has in hand several normal prostate epithelial cells and prostate cancer cells and I will now focus my efforts on these cells. In Aim 1, we found that pericentrin truncations lacking the PKA binding domain decrease the ability of pericentrin to induce spindle abnormalities and aneuploidy. We are currently making single point mutations in this domain to rule out nonspecific effects of protein truncation and we are also making similar truncations and mutations in the PKC and PKB binding domains of pericentrin.

Work on Aim 2 has yielded new and exciting results and we are now focusing most of our efforts here. In Aim 2 we are testing the effect of altering pericentrin levels in cells with alterations in levels of other oncogenes and tumor suppressors. We had previously shown that an increase in pericentrin levels induces an increase in centrosome number (Pihan et al, 2001). We now show that depletion of pericentrin by RNA interference (Fig. 1, using small interfering RNAs, siRNAs, Elbashir et al, 2001) induced the opposite effect, loss of centrioles/centrosomes, as well as defects in centrosome structure (centriole separation, Fig. 2). Moreover, these structural defects occurred together with functional changes in centrosomes including loss of primary cilia and reduced ability to duplicate centrosomes (Fig. 3). Primary cilia are solitary cilia that function as sensors (e.g. rods, cones) and are implicated in cell division control (Pazour and Witman, 2003). The functional changes induced by pericentrin depletion were also the opposite of pericentrin overexpression, which results in “overduplication” of centrosomes (Pihan et al, 2001). Thus, it appears that modulating pericentrin levels can control centrosome number and centrosome integrity, two parameters that are likely to be important for cell

viability (centrosome duplication and integrity) and tumor potential (excess centrosomes, which can lead to multipolar spindles, chromosome instability and aneuploidy).

Upon further investigation we found that pericentrin depletion induced cell cycle arrest in normal human diploid cells (Fig. 4). This suggested a mechanism (cell cycle arrest) for preventing the deleterious downstream consequences of centrosome 'damage' and centrosome dysfunction. These data suggest the presence of a new checkpoint that monitors 'centrosome damage' just as the DNA damage checkpoint monitors DNA damage. The presumed checkpoint has all the expected elements. It is initiated by a defect (centrosome damage) that induces a signal (transducer, p53) and a possible receiver (p38).

In another set of experiments and to our surprise (unintentionally actually), we found that cells with functionally inactive or altered p53 suppressed the cell cycle arrest induced by pericentrin depletion (Fig. 5). This allowed the cells to cycle in the presence of centrosome 'damage'. This suggested that cells that slip through the checkpoint might suffer the consequences of centrosome damage.

To test this, we examined p53-deficient cells at different time periods after RNAi treatment. Two days after RNAi treatment we found cells that often lacked centrosomes at spindle poles. The cells accumulated in metaphase with monopolar spindles (as expected if centrosomes are damaged or lost and cannot make a bipolar spindle). To further examine the ultimate consequences of centrosome and spindle defects we looked at longer times after siRNA treatment, something we do not typically do. For this reason, we had to split and replate cells and retreat with siRNAs targeting pericentrin. We found that at these later times (>48hours), there was a significant decrease in the number of pericentrin siRNA treated cells compared with control siRNA treated cells. Upon closer inspection, we found that the cells appeared to be dying as they were rounding up and detaching from the plate. In fact, through the use of an apoptotic marker (M30) we could show that there was a ~5-fold increase in apoptosis in pericentrin siRNA treated cells (Fig. 6). Subsequently, we showed that p38 inhibitors suppressed the cell cycle arrest, suggesting that the p38 stress activated kinase is required for this checkpoint, in addition to p53. We are now testing other molecules (tumor suppressors, oncogenes, regulatory proteins) that are known to be involved in or associated with prostate cancer (e.g. PTEN, Akt) to see if they suppress the G1 arrest and thus induce cancer-cell-specific cell death. We will test whether pericentrin depletion acts antagonistically with p53 and p38 and other prostate associated molecules in the induction of cell cycle arrest in normal prostate epithelial cells (gift of Dr. William Hahn, see Berger et al, 2004) and p53 wild type cell lines (e.g. LNCaP) and in the induction of apoptotic death in prostate tumor cells with abrogated p53 (PC3, DU-145). This work identifies a potentially novel pathway for treating prostate cancer, namely depletion of pericentrin in cells with abrogated regulatory pathways (p53, p38) and potentially other pathways (PTEN, AKT).

Key research accomplishments

*Pericentrin depletion causes centrosome defects and centrosome loss.

*Pericentrin depletion causes loss of primary cilia and blocks centrosome duplication (centrosome over-duplication is implicated in supernumerary centrosomes and aneuploidy in prostate cancer)

*Pericentrin depletion arrests diploid cells in G1.

*Pericentrin induced cell cycle arrest is p53-dependent.

* Pericentrin induced cell cycle arrest seems to be p38 dependent.

*This work identifies a potentially a new checkpoint that monitors “centrosome damage”.

* p53-deficient cells continue to cycle in the presence of structural and functional defects in centrosomes.

*p53-deficient cells ultimately die by apoptosis as a result of centrosome and spindle defects.

*****These results suggest a cancer-cell-specific treatment for prostate cancer. Pericentrin depletion would kill cells with abrogated p53 function but would spare normal prostate epithelial cells. The latter would arrest until the ‘centrosome damage’ is repaired (e.g. after the pharmacological treatment of patients with pericentrin inhibitors).

Reportable outcomes

*Constructed GFP-centrin cell lines (RPE, HeLa) to study centrosome duplication (I plan to make similar lines in prostate epithelia and tumor cells)

*Produced GFP-gamma tubulin cell lines (RPE, HeLa) to study centrosome duplication (I plan to make similar lines in prostate epithelia and tumor cells)

*Produced a GFP-IFT20 HeLa cell line to study primary cilia formation.

Conclusions

During the last year, we have made two important discoveries. The first is that we identified a potentially new checkpoint that monitors loss of centrosome integrity. Like DNA damage, this checkpoint is overcome when molecules that control the pathway (p53, p38) are depleted or inhibited. The e second discovery is that cells that lack p53, p38 and likely other regulatory molecules *do not* undergo cell cycle arrest. Slippage through the centrosome damage checkpoint may provide a unique door into combating prostate cancer. It may provide a prostate cancer cell specific mechanism for eliminating prostate cancers, in that normal prostate epithelial cells will arrest in the cell cycle while tumor cells deficient for p53, p38 and perhaps other prostate cancer specific molecules of the pathway will die when pericentrin is depleted.

References

Doxsey S, McCollum D, Theurkauf W. Centrosomes in cellular regulation. Annu Rev Cell Dev Biol. 2005;21:411-34.

Berger R, Febbo PG, Majumder PK, Zhao JJ, Mukherjee S, Signoretti S, Campbell KT, Sellers WR, Roberts TM, Loda M, Golub TR, Hahn WC. Androgen-induced differentiation and tumorigenicity of human prostate epithelial cells. Cancer Res. 2004 Dec 15;64(24):8867-75.

Lingle WL, Lutz WH, Ingle JN, Maihle NJ, Salisbury JL. Centrosome hypertrophy in human breast tumors: implications for genomic stability and cell polarity. Proc Natl Acad Sci U S A. 1998 Mar 17;95(6):2950-5.

Pazour GJ, Witman GB. The vertebrate primary cilium is a sensory organelle. Curr Opin Cell Biol. 2003 Feb;15(1):105-10.

Pihan GA, Purohit A, Wallace J, Knecht H, Woda B, Quesenberry P, Doxsey SJ. Centrosome defects and genetic instability in malignant tumors. Cancer Res. 1998 Sep 1;58(17):3974-85.

Pihan GA, Purohit A, Wallace J, Malhotra R, Liotta L, Doxsey SJ. Centrosome defects can account for cellular and genetic changes that characterize prostate cancer progression. Cancer Res. 2001 Mar 1;61(5):2212-9.

Pihan GA, Wallace J, Zhou Y, Doxsey SJ. Centrosome abnormalities and chromosome instability occur together in pre-invasive carcinomas. Cancer Res. 2003 Mar 15;63(6):1398-404.

Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature. 2001 May 24;411(6836):494-8.

Zimmerman WC, Sillibourne J, Rosa J, Doxsey SJ. Mitosis-specific anchoring of gamma tubulin complexes by pericentrin controls spindle organization and mitotic entry. Mol Biol Cell. 2004 Aug;15(8):3642-57.

APPENDIX

Fig. 1

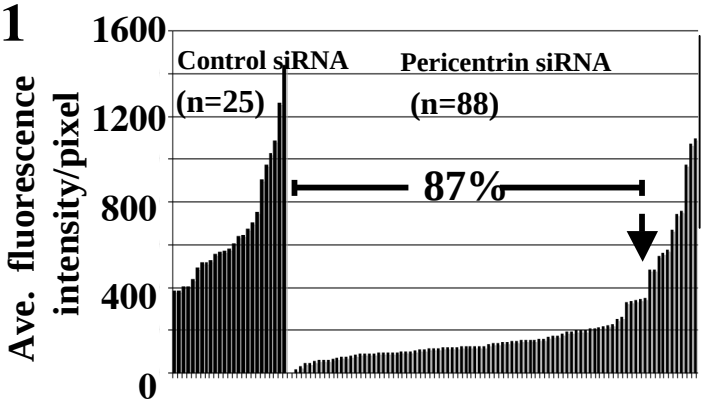
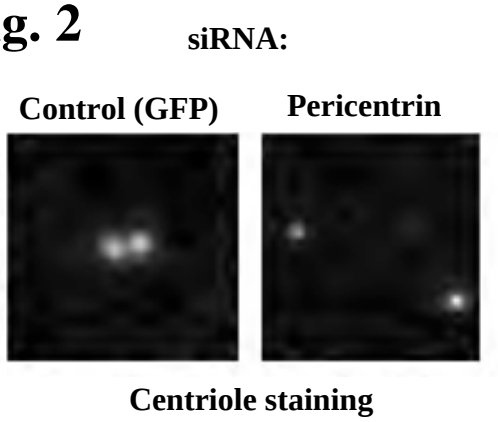


Fig. 2



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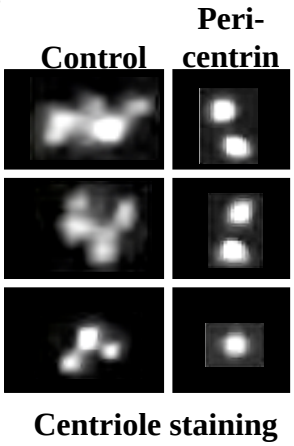
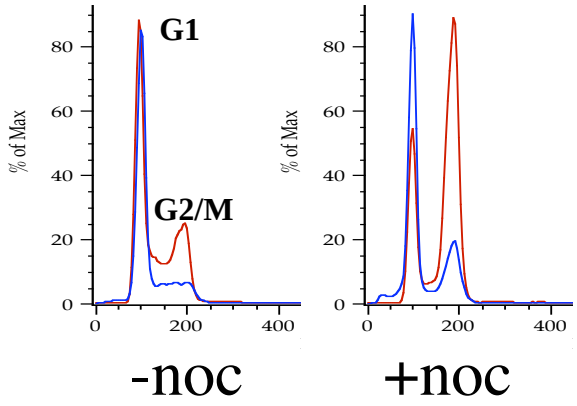


Fig. 4



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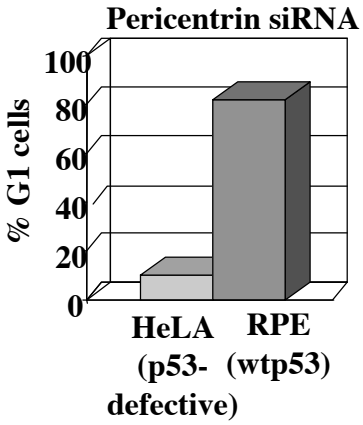

Fig. 6

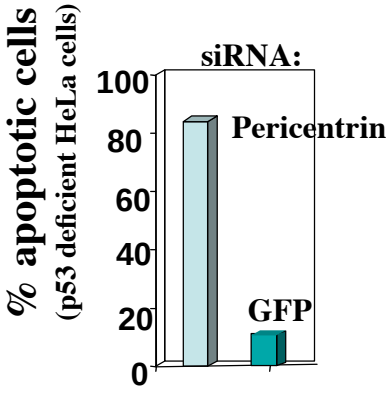

Figure Legends. Fig. 1. Pericentrin depletion induces loss of the protein at centrosomes. 87% of the pericentrin siRNA treated cells have centrosome intensities that are less than the lowest level in controls (lamin). Each bar represents the fluorescence intensity of one centrosome. **Fig. 2.** Immunofluorescence staining of the two centrioles of the centrosome showing separation in pericentrin depleted cells (right, 25.7% of cells) compared with tight association in controls (left, only 3.5% separated). **Fig. 3.** U2OS cells treated with hydroxyurea show expected centrosome overduplication in control cells (left, multiple clustered dots, GFP siRNA) compared with a reduced number in pericentrin siRNA treated cells (right). **Fig. 4.** Flow cytometeric analysis showing that pericentrin depleted cells (blue) do not shift from the G1 peak (-noc) to the G2/M peak in the presence of nocodazole (+noc) whereas control cells do (red, +noc). **Fig. 5.** p53 deficient cells do not arrest in G1. **Fig. 6.** The percentage of apoptotic cells is dramatically higher in pericentrin siRNA treated cells deficient in p53 (HeLa) whereas control cells (GFP siRNA treated) have a lower percentage of apoptotic cells.